TITLE:
Designed poly(ethylene glycol)/poly(D,L-lactide)-based hydrogel structures prepared by stereolithography

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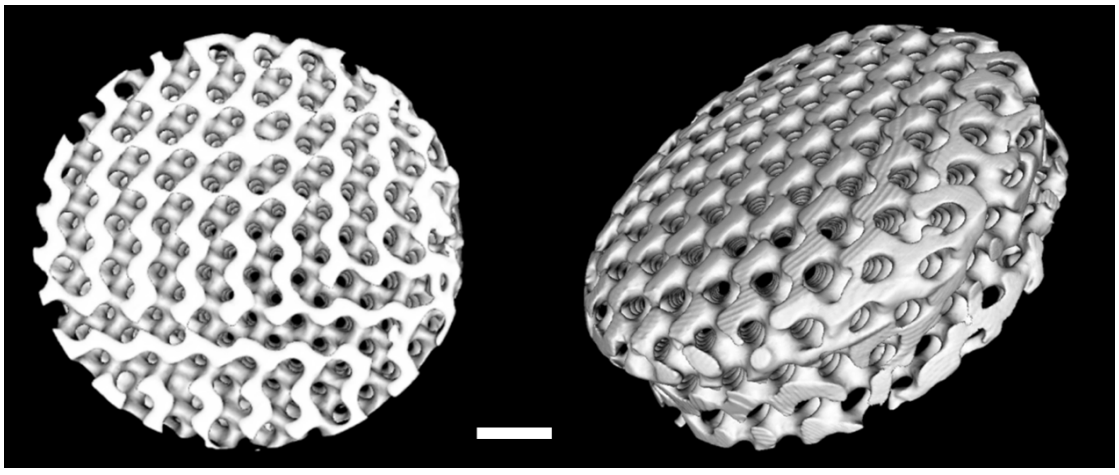
ABSTRACT SUMMARY:
Three-dimensional biodegradable poly(ethylene glycol)/poly(D,L-lactide) hydrogel structures were prepared by stereolithography. A photo-polymerisable liquid resin comprising PDLLA-PEG-PDLLA-based macromer, visible light photo-initiator, dye and inhibitor in DMSO/water was used to build the structures. Hydrogels with well-defined architectures and good mechanical properties were prepared. Hydrogel structures with a gyroid pore network architecture showed narrow pore size distributions, excellent pore interconnectivity and good mechanical properties. The structures showed good cell seeding characteristics, and human mesenchymal stem cells adhered and proliferated on these materials.

INTRODUCTION:
Of all rapid prototyping methods, stereolithography is the most developed technique with the highest accuracy. Three-dimensional designed constructs can be precisely fabricated in a layer-by-layer manner. Its working principle is based on the spatially controlled solidification of a liquid photo-polymerisable resin upon illumination with a computer-driven light source. Poly(D,L-lactide)-based resins for tissue engineering applications have been used for this.[1,2] Hydrogel structures prepared from poly(ethylene glycol) based resins have also been fabricated using stereolithography.[3-5] Although these structures showed good biocompatibility and the PEG hydrogels could be used to encapsulate living cells, they are not degradable. By block copolymerisation of these two polymers, hydrogels with biodegradable and biocompatible characteristics can be obtained.[6]
In this paper we describe the synthesis of PDLLA-PEG-PDLLA-based macromers, the resin formulation, and the photo-polymerisation process by stereolithography that allows the generation of designed three-dimensional crosslinked structures.[7,8] The obtained networks are characterised and seeded with mesenchymal stem cells, to evaluate cell adhesion and proliferation behaviour.

EXPERIMENTAL METHODS:
PDLLA-PEG-PDLLA oligomers with a number average molecular weight of approximately 4600 g/mol were prepared by ring opening polymerisation of D,L-lactide initiated from the hydroxyl groups of a PEG 4k diol using $Sn(Oct)_2$ as a catalyst. Functionalization with methacrylic ester groups was subsequently done by reaction with methacrylic anhydride.
A liquid photo-polymerisable resin suitable for use in stereolithography was then formulated. This mixture contained 33 wt% MA-DLLA-PEG-DLLA-MA macromer, 1.7 wt% photo-initiator (Lucirin TPO-L), 0.19 wt% phenol red dye, 0.1 wt% hydroquinone,

and 11 wt% DMSO and 54 wt% water. The resin was used to prepare biodegradable hydrogel structures with a commercially available stereo-lithography apparatus (EnvisionTec Perfactory Mini Multilens). Disk-shaped porous hydrogel structures having a gyroid pore network architecture [2] and solid specimens were prepared. Structural analysis of the porous scaffolds was done by micro-computed tomography (μCT, GE eXplore Locus SP operating at 14 μm resolution) after conditioning the specimens in the ambient environment.
The attachment, morphology, distribution and proliferation of human mesenchymal stem cells (hMSCs) was evaluated by light microscopy and scanning electron microscopy after seeding in and on the hydrogel films and structures and cell culturing for 1 d and 5d.

RESULTS AND DISCUSSION:
Hydrogels in the form of solid disks and porous scaffolds were obtained by stereolithography upon by irradiation of aqueous solutions of PDLLA-PEG-PDLLA-based macromers with a blue light source. The structures were extracted with distilled water to remove unreacted water-soluble compounds. The built hydrogel structures showed high flexibility and well-defined architectures, accurately matching the design.
The gyroid architecture is mathematically defined, and allows precise control of the porosity and pore size of a fully interconnected pore network. This is of great importance In tissue engineering, as homogeneous distribution of cells and adequate transport of nutrients and waste products throughout the scaffold is ensured.
Micro-computed tomography allows assessing structural parameters such as porosity and the pore size distribution of the pore network inside scaffolds. The figure depicts μCT images of two porous gyroid hydrogel scaffolds. This architectural design and the stereolithography technique allow a precise control of porosity and pore size of a fully interconnected pore network, as can be clearly seen in the images.



Images constructed from μCT scans of hydrogel scaffolds prepared by stereolithography from PDLLA-PEG-PDLLA based resins. Scale bar is 1 mm.

The porosity of the structures was 52 %, while the porosity in the designed architecture was 55 %. As result of the regularity of the design and the precision of the building technique employed, the pore size distribution was quite narrow. The average pore size was 423 μm, while pores ranging in size from 387 to 558 μm constituted more than 76 % of the pore volume. The obtained hydrogel scaffolds showed excellent pore interconnectivity.

In order to evaluate the cell adhesion behaviour, (hMSCs) were seeded on hydrogel films and in/on porous hydrogel scaffolds fabricated by stereolithography. Although PEG is known to limit the adhesion of cells to surfaces, we did observe the attachment of hMSCs to our PDLLA-PEG-PDLLA hydrogels after 1 d of culturing. (It should be noted that the hydrogel structures were incubated in culture medium containing 10 % FBS overnight before cell-seeding.) It seemed that after 5 d the cells had a tendency to aggregate. It has been shown that in the absence of integrin-binding ligands, cells undergo an apoptotic progression due to lack of matrix interactions.[9] To ensure better cell attachment and cell proliferation, cell-binding ligands, such as peptides with the RGD sequence, should be incorporated into the structures. This can be done through Michael type additions to unreacted double bond functionalities at the network surfaces. In any case, we have shown the potential of these built PDLLA-PEG-PDLLA-based hydrogel structures for use in tissue engineering and cell culturing.

CONCLUSIONS:

The application of stereolithography for the fabrication of designed porous and solid hydrogel structures using aqueous solutions of photo-crosslinkable biodegradable materials based on poly(ethylene glycol)/poly(D,L-lactide) macromers and a visible light photo-initiator was demonstrated. Network structures with well-defined architectures having narrow pore size distributions and high pore interconnectivities as well as good mechanical properties were obtained.
Seeded human mesenchymal stem cells attached to the hydrogel materials and remained viable for 5 days.

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